Drug and Alcohol Dependence 133 (2013) 789–793

Contents lists available at ScienceDirect

Drug and Alcohol Dependence

journal homepage: www.elsevier.com/locate/drugalcdep

Short communication

Differential expression and functional role of cannabinoid genes in alcohol users

Marisela Agudelo*, Adriana Yndart, Marisol Morrison, Gloria Figueroa, Karla Muñoz, Thangavel Samikkannu, Madhavan P. Nair

Florida International University, United States

ARTICLE INFO

Article history:
Received 28 February 2013
Received in revised form 17 June 2013
Accepted 24 August 2013
Available online 5 September 2013

Keywords:
Alcohol
Cannabinoids
Cannabinoid receptors
Human dendritic cells

ABSTRACT

Background: Genetic factors account for about fifty percent of the risk for alcoholism and alcohol dependence (AD) has been reported to be influenced by cannabinoid receptors (CBRs) and the endocannabinoid system (ECS). Previous studies have focused on cannabinoids and alcohol-related effects in the CNS; however, the role CBRs play on alcohol effects in the immune system has not been elucidated yet. Since alcohol can affect immune responses and have detrimental effects on immune cells such as dendritic cells (DCs), we hypothesize that alcohol can exert its effects on DCs by modulating changes in CBRs, which in turn can regulate important DC functions such as cytokine production.
Methods: Therefore, we studied the expression of CNR1 and CNR2, and the novel cannabinoid G protein-coupled receptor (GPCR) 55 (GPR55) in human monocyte-derived dendritic cells (MDDCs) from alcohol users. CNR1, CNR2, and GPR55 genes were measured by qRT-PCR and protein by flow cytometry. MDDCs from alcohol users show significantly higher levels of CNR2 and GPR55 compared to MDDCs from non-users. These findings were further confirmed using MDDCs treated with alcohol. Inflammatory cytokines were measured in EtOH-treated and non-treated cells by antibody array.
Results: Functional effects of CBRs on MDDCs were shown by CB2 and GPR55 siRNA transfection. Transfected EtOH-treated cells showed significantly higher levels of proinflammatory cytokine production as measured by IL-1β expression. Our results provide insights into alcohol mechanisms of DC regulation and show, for the first time, that alcohol is inducing CNR2 and GPR55 in human DCs.

Published by Elsevier Ireland Ltd.

1. Introduction

According to NIAAA, 18 million Americans suffer from alcohol use disorders (AUDs; NIAAA, 2007), and genetic factors account for fifty percent of the risk for alcoholism (Foroud et al., 2010; Schuckit, 2009). Molecular and behavioral studies confirm a critical role of the innate immune system in mediating the effects of alcohol and support a neuroimmune hypothesis of alcohol addiction (Mayfield et al., 2013). Although it has been known that alcohol interferes with immune functions, and as a result, alcoholics may be more susceptible to infections (Szabo, 1997); to date, one of the least appreciated medical consequences of AUDs is their effects on the immune system leading to a growing demand for research to elucidate the molecular mechanisms of alcohol-induced immunomodulation. Therefore, our study aims to elucidate the effects of alcohol on human dendritic cells (DCs) which are pivotal antigen presenting cells of the innate immune system.

Alcoholism is a complex system influenced by CBRs (Pava and Woodward, 2012), and the ECS has been shown to be involved in mechanisms underlying AD (Erdozain and Callado, 2011; Marcos et al., 2012). The neurological effects of cannabinoids are mainly reported to be associated with CB_1 (Gaffuri et al., 2012) while the peripheral actions to be mediated by CB_2 (Miller and Stella, 2008). However, both receptors are known to play major roles in CNS (Mechoulam and Parker, 2013) and periphery (Kaplan, 2012). CB_2 activation, in particular, has a potential role in neuroinflammation and immunomodulation as recently reviewed (Rom and Persidsky, 2013). Overall, CBRs are known to have immunomodulatory properties; for instance, murine studies have reported a role of CBRs in DC regulation during inflammation (Lu et al., 2006a,b; Newton et al., 2004). In terms of CB_2, for instance, agonists have been reported as potent anti-inflammatory and neuroprotective agents with beneficial effects of CB_2 activation in the case of concomitant HIV infection and alcohol abuse (Persidsky et al., 2011). Other reports from human brain tissues and microvascular

* Corresponding author at: Department of Immunology, College of Medicine, AHC-I 417A, Florida International University, 11200 SW 8th Street, Miami, FL 33199, United States. Tel.: +1 305 348 6503; fax: +1 305 3481109.
E-mail address: magudelo@fiu.edu (M. Agudelo).

0376-8716/$ – see front matter. Published by Elsevier Ireland Ltd.
http://dx.doi.org/10.1016/j.drugalcdep.2013.08.023

endothelial cells have demonstrated that CB_2 is highly up-regulated during inflammation (Ramirez et al., 2012). However, most of the studies correlating CBRs to AUDs, have been done in the context of other inflammatory disorders in the CNS. Therefore, the alcohol-induced alterations of CBRs and their functional role in immunomodulatory mechanisms are still unclear. Moreover, there is evidence that additional CBRs may exist such as the novel GPR55 (Ryberg et al., 2007); which has been suggested to be involved in metabolism and glucose homeostasis (Romero-Zerbo et al., 2011); and in inflammatory responses mediated by neutrophils (Balenga et al., 2011).

Since alcohol is known to impair innate immune cell function (Szabo, 1997; Szabo et al., 2012), CBRs have been reported to regulate DCs (Kaplan, 2012), and the ECS is involved in AUDs (Erdozain and Callado, 2011), we hypothesize that alcohol can exert its effects on DCs by modulating CBRs, and subsequently CBRs may alter DC functions. We are the first ones to report the effects of alcohol drinking on CBRs produced by MDDCs and to show a functional role of CB_2 and GPR55 on cytokine production using a siRNA transfection approach. Analyzing the functional role of CBRs in alcohol-induced modulation of MDDCs will contribute to the current literature that steers toward a major role of the ECS in AUDs (Pava and Woodward, 2012).

2. Materials and methods

2.1. Participants

Blood donors were recruited from the Borinquen Health Care Center, Inc., Miami. Consents were obtained consistent with Florida International University (FIU) and the National Institutes of Health (NIH) policies. The protocol was approved by the IRB of FIU. Exclusion criteria were polydrug use, Hepatitis, HIV, other medical conditions, age <18 and >52 years, and pregnancy. Prior to enrollment in the study, alcohol users ($n = 7$) and non-users ($n = 7$) completed a questionnaire. Alcohol users reported drinking an average of 5 days/week and an average of 7 drinks/day. Race/ethnicity of the participants includes African American, White, and Hispanics. Participants' average age is 35.

2.2. Differentiation of MDDCs

Blood samples from alcohol users and non-users were drawn. MDDCs were prepared from peripheral blood mononuclear cells (PBMCs) as previously described by us (Nair et al., 2005, 2009).

2.3. Gene expression by quantitative real-time PCR (qRT-PCR)

Gene expression was assessed as previously described by us (Agudelo et al., 2011, 2012) using Taqman assays (Applied Biosystems) for the expression of CB_1 (Hs00275634_m1), CB_2 (Hs00275635_m1), GPR55 (Hs00995276_m1), and IL-1β (Hs01555410_m1). GAPDH (Hs99999905_m1) and 18S rRNA (catalog # 4333760F) were used as endogenous controls.

2.4. Analysis of intracellular CB_1, CB_2 and GPR55 by flow cytometry

After MDDC differentiation, the cells were treated with 10–40 mM EtOH for 24 h, harvested, blocked, fixed, and permeabilized with cytofix/cytoperm (BD Bioscience). CB_1 (catalog # sc-20754), CB_2 (catalog # sc-25494), and GPR55 (catalog # 10224) proteins were detected with primary rabbit-polyclonal (Santa Cruz Biotechnology, Santa Cruz, CA; and Cayman Chemicals, Ann Arbor, MI respectively) and secondary goat anti-rabbit IgG fluorescein (FITC)-conjugated (Millipore) antibodies. Cells were acquired on an Accuri C6 flow cytometer (BD Accuri; Ann Arbor, MI) and analyzed with FlowJo software (Tree Star, Inc., Ashland, OR).

2.5. Cytokine array

Non-treated and treated cells were harvested and whole cell lysates were prepared in protein extraction reagent (Pierce Biotechnology, Rockford, IL). 50 μg of protein were added to the array membranes (catalog # AAH-INF-3-8, RayBiotech, Norcross, GA). The intensities of signals were quantified by densitometry using Image J software. Data were analyzed using the RayBio Analysis Tool.

2.6. siRNA transfection against CNR2 and GPR55

CNR2 and *GPR55* silencing was performed using siRNA transfection kit following manufacture's protocol. (Santa Cruz Biotechnology, catalog # sc-45064) along with control (catalog # sc-37007), CB_2 (catalog # sc-39912) or GPR55 siRNA (catalog # sc-75183). Gene silencing was confirmed by qRT-PCR.

3. Results

3.1. MDDCs from alcohol users and treated with EtOH have higher levels of CNR2 and GPR55

MDDCs from alcohol users (Fig. 1a) show significantly higher *CNR2* and *GPR55* compared to MDDCs from controls; and there were no significant effects on *CNR1*. And when MDDCs were treated with alcohol (0.05%, 0.1% and 0.2%), there was an increased and bell curved pattern in *CNR2* and *GPR55*, with a more significant effect at the 0.1% alcohol concentration (Fig. 1b).

3.2. MDDCs treated with EtOH have higher levels of CB_2 and GPR55 protein

MDDCs were treated with alcohol (0.1% and 0.2%) for 24 h and analyzed by flow cytometry. Accordingly, *in vitro* protein results (Fig. 1c) correlate with the gene data showing an increased in CB_2 and GPR55.

3.3. EtOH induces up-regulation of inflammatory cytokines

Since one of the main functions of DCs is the production of cytokines and there is evidence that alcohol-induced inflammation is mediated by proinflammatory cytokines (Lippai et al., 2013), we measured and compared the levels of cytokines in untreated and EtOH-treated MDDCs (Fig. 2a) using antibody array (RayBiotech). EtOH significantly up-regulated 19 cytokines compared to the untreated control (Supplementary Table 1). Cytokine levels in the EtOH-treated cells ranges from 2.3 to 18.6 folds higher than untreated cells.

3.4. CB_2 and GPR55 siRNA transfection

In order to establish potential mechanisms of receptor up-regulation in the context of alcohol exposure, we used siRNA against CB_2 and GPR55 to analyze the effect of gene silencing on MDDCs function. As confirmed by qRT-PCR, CB_2 and GPR55 were significantly knockdown compared to the untransfected controls and EtOH-treated samples, and siRNA transfected controls (Fig. 2b).

3.5. Functional effects of CB_2 and GPR55 siRNA transfection on cytokine production

After confirming the CB_2 and GPR55 transfections were successful, cytokine levels were measured in untransfected and transfected controls and EtOH-treated MDDCs. Surprisingly, CB_2 and GPR55 transfected EtOH-treated cells show significantly higher levels of IL-1β (>20 fold difference) compared to the untransfected EtOH-treated cells (Fig. 2c).

3.6. Statistics

Data were analyzed using GraphPad Prism software. Comparisons between groups were performed using paired *t*-test and two-way ANOVA. Differences were considered significant at $p \leq 0.05$. Data are expressed as mean ± SEM. Experiments were performed at least three times in triplicates. Arrays were

Download English Version:

https://daneshyari.com/en/article/10509601

Download Persian Version:

https://daneshyari.com/article/10509601

Daneshyari.com